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than a corresponding wild type plant under non-heavy metal conditions.

23. (New) A method according to claim 16 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.

24. (New) A method according to claim 17 wherein the plant grows not significantly differently than a corresponding wild type plant under non-heavy metal conditions.

REMARKS

The claims are amended to address the definiteness issues raised by the Examiner. The terms unengineered and untransformed are used interchangeably, see p. 11, line 10. New claims 20-24 require that the plant exhibit otherwise normal growth; that the transgenic plants are phenotypically the same as wild type plants and have the same growth characteristics finds support on p.11, lines 9-10 and lines 28-29. These amendments introduce no new matter.

35USC112, second paragraph.

The claims are believed to be definite. Applicants have adopted all the amendments kindly suggested by the Examiner.

35USC102(b)

The claims require a plant which is genetically engineered to overexpress glutamylcysteine synthetase *and thereby provides enhanced heavy metal accumulation*. The Action suggests that the poplars of Arisi et al. might inherently provide enhanced heavy metal accumulation. However, Arisi's subsequent report demonstrates that these poplars do not provide enhanced heavy metal accumulation, i.e. they showed no increased heavy metal accumulation compared with untransformed controls (Noctor et al., p.640, col.1, last paragraph - cited on p.3, line 9 of our Specification and enclosed herewith). As Arisi's poplars do not provide "enhanced heavy metal accumulation" they do not and can not meet or suggest the limitations of our claims.

Raskin et al. suggests that plants engineered to express high levels of metallothioneins (MT's) could be used for phytoremediation of metals. Watanabe et al. describes an *E. coli* gamma-glutamylcysteine synthetase (ECS) gene and Chen et al. (1994) report that mutant tomato cells selected for cadmium tolerance show increased ECS activity. The issue then, is whether the cited art suggests modifying Raskin to overexpress ECS, rather than an MT, and thereby secure a plant providing enhanced heavy metal accumulation.

A detailed reading of Chen and subsequent work from Chen's laboratory (not cited in the Action) reveals that the prior art not only fails to suggest the claimed invention, but in fact teaches directly away from it. In their discussion section, Chen acknowledges that the relationships between ECS activity, glutathione synthetase (GS) activity, phytochelatin (PC) synthesis, heavy metal tolerance and heavy metal accumulation are by no means clear. While Chen's results are similar to those of Steffens et al. (1989), cited by Chen on p.238 col 1, lines 50-53, other published reports suggest the opposite. For example, at p.238, col 2, line 20-25 Chen also cites de Knecht et al. (1992) for demonstrating that Cd-tolerant plants can synthesize fewer PCs than sensitive plants exposed to the same Cd concentration. Other data cited by Chen suggest that this mechanism of Cd-tolerance may not provide a practical route for generating useful plants. First, Chen's Cd-tolerance is not stable (Chen, p.238, col 1, lines 12-14) and second, such metal tolerant plants demonstrate poor growth characteristics (Chen, p.238, col 1, lines 22-25). Chen concludes by suggesting that future development of transgenic plants with altered capacities to synthesize either GSH or PCs might be used to test their hypothesis that increased GSH and/or PC synthesis increases Cd tolerance.

The senior author of Chen et al. subsequently reported on exactly these experiments (see our Specification, p.3, lines 5-9 and the Goldsbrough, 1999, reference cited therein enclosed herewith) and like Arisi's poplars, Goldsbrough's transformed *Arabidopsis* plants provided no increase in heavy metal accumulation compared with controls. Specifically, Goldsbrough reports that while ECS could restore some degree of Cd tolerance to a Cd-sensitive mutant (a *cad2* mutant having reduced GSH levels), this gene did not increase Cd tolerance of wild type plants (Goldsbrough, p.230, line 35). Interestingly, Goldsbrough also further confounds the teachings

of Chen by reporting that the ECS gene does not show any change in RNA expression in plants or cells that are exposed to Cd (Goldsbrough, p.230, lines 28-30).

The prior art does not suggest modifying Raskin to overexpress ECS, rather than an MT, and thereby secure a plant providing enhanced heavy metal accumulation. The prior art establishes an uncertain and unpredictable relationship between ECS expression and heavy metal accumulation, and specifically teaches (in both Noctor et al. and Goldsbrough) that over expression of ECS will not yield heavy metal accumulators. Note additionally that the "normal growth" requirement of new claims 20-24 is specifically discouraged by Chen at p.238, col 1, lines 22-26 ("Metal-tolerant plants have also been shown to produce less biomass and have reduced fitness compared to their nontolerant counter-parts when grown in normal soil").

Absent a prior art suggestion to modify Raskin's metallothionein transformed plants to overexpress glutamylcysteine synthetase and thereby provide enhanced heavy metal accumulation - as required by all the pending claims - the claims are submitted to be in compliance with 35USC103(a).

Applicants hereby petition for any necessary extension of time pursuant to 37 CFR 1.136(a). The Commissioner is hereby authorized to charge any necessary fees (small entity) or credit any overcharges associated with this communication to our Deposit Account No. 19-0750 (order no.B99-085).

Respectfully submitted,
SCIENCE & TECHNOLOGY LAW GROUP



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enc. Goldsbrough, 1999, Metal tolerance in plants: the role of phytochelatins and metallothioneins. *In* N Terry, GS Banuelos, eds, Phytoremediation of Trace Elements. Ann Arbor Press, Ann Arbor, MI), 7 pg.

Noctor et al., 1998, J Exp Bot 49:523-647, 25 pg.